REMARKS

Claims 1 and 5 have been amended. Claims 1-5 are now pending in this application. Support for the amendments is found in the existing claims and the specification as discussed below. Accordingly, the amendments do not constitute the addition of new matter. Applicant respectfully requests the entry of the amendments and reconsideration of the application in view of the amendments and the following remarks.

Telephonic interview

Applicant's representative thanks Examiner Shaw and her Supervisor, Mr. Ram Shukla, for the helpful interview conducted by telephone in November 30, 2006 which is summarized herein.

Rejection under 35 U.S.C. § 112, second paragraph

Claims 1-4 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner considers that the phrase "under conditions in which the first probe and the second probe are annealed and the first probe and the target nucleic acid are annealed" is open to different interpretations. With this amendment, the above phrase has been deleted.

In addition, as discussed in the interview of November 30th, two additional steps are added which spell out what is happening when the components are mixed.

Step (b) recites that a loop forms and that as a result of forming the loop, the signal is quenched.

Step (c) recites that the sample is added and the target anneals with the first probe.

The final clause states "wherein the signal is quenched when the first probe and the second probe are annealed and not quenched when the first probe and the second probe are not annealed, in the presence of the target" which summarizes what happens when the target is present versus when the target is not present and the two probes anneal to each other.

Support for the amendments is described below.

The phrase "wherein the nucleic acid of the second probe is labeled with a labeling material generating a signal by which formation of the loop can be detected" is supported by the language of original claim 1. This phrase has merely been moved for better clarity.

Support for the inclusion of "(b) forming the loop in the loop region when the second probe is annealed with the first probe, thereby quenching the signal from the labeling material in the absence of the target;" is found in the specification at page 8, lines 5-9; page 9, line 5 to page 10, line 3; page 10, lines 13-17. On page 14, lines 13-14, it is stated that "with Probe 2 only, fluorescence was observed but in the case where Probe 1 was added, the fluorescence was quenched considerably", thus providing support for the recitation that the signal is quenched when the second probe is annealed with the first probe.

Support for "(c) adding the sample, whereby the target nucleic acid in the sample anneals with the first probe;" is found in the specification at page 7, lines 19-20 which states that Probe 1 hybridizes with the target and page 11, first paragraph.

Support for the limitation that "a higher intensity in the signal of the labeling material [is detected] in the presence of the target," is found in the last paragraph on page 14 which discloses that a higher intensity of fluorescence was observed when the target was present.

Support for the limitation that "the signal is quenched when the first probe and the second probe are annealed and not quenched when the first probe and the second probe are not annealed, in the presence of the target" is found on page 9, last 5 lines.

In view of Applicant's amendments, reconsideration and withdrawal of the above ground of rejection is respectfully requested.

Rejection under 35 U.S.C. § 102(a) and (e)

Claims 1 and 3-5 remain rejected under 35 U.S.C. § 102(a) and (e) as anticipated by Weston, et al. (US Patent 6391593).

The Office Action asserts that the claims do not preclude the possibility that the first and second probes and target could all bind together as in Weston. The amendment to claim 1 addresses this point. Support for the amendments to claim 1 is reiterated below.

The phrase "wherein the nucleic acid of the second probe is labeled with a labeling material generating a signal by which formation of the loop can be detected" is supported by the language of original claim 1. This phrase has merely been moved for better clarity.

Support for the inclusion of "(b) forming the loop in the loop region when the second probe is annealed with the first probe, thereby quenching the signal from the labeling material in the absence of the target;" is found in the specification at page 8, lines 5-9; page 9, line 5 to page

10, line 3; page 10, lines 13-17. On page 14, lines 13-14, it is stated that "with Probe 2 only, fluorescence was observed but in the case where Probe 1 was added, the fluorescence was quenched considerably", thus providing support for the recitation that the signal is quenched when the second probe is annealed with the first probe.

Support for "(c) adding the sample, whereby the target nucleic acid in the sample anneals with the first probe;" is found in the specification at page 7, lines 19-20 which states that Probe 1 hybridizes with the target and page 11, first paragraph.

Support for the limitation that "a higher intensity in the signal of the labeling material [is detected] in the presence of the target," is found in the last paragraph on page 14 which discloses that a higher intensity of fluorescence was observed when the target was present.

Support for the limitation that "the signal is quenched when the first probe and the second probe are annealed and not quenched when the first probe and the second probe are not annealed, in the presence of the target" is found on page 9, last 5 lines. Claim 5 has also been amended to include this limitation.

Weston, et al. do not teach all of the limitations of the present claims. For example, Weston, et al. do not teach "(b) forming the loop in the loop region when the second probe is annealed with the first probe, thereby quenching the signal from the labeling material in the absence of the target". Weston, et al. specifically teach that the first and second probes should not anneal in the absence of the target. As discussed in col. 3, lines 20-23, Weston, et al. teach the introduction of "a destabilizing moiety that prevents the two oligonucleotide probes from associating in the absence of the target nucleic acid and hence reducing noise from the potential association of these probes". In contrast to Applicant's claimed invention, Weston, et al. do not want the two probes to anneal to each other in the absence of the target sequence. This is opposite to the presently claimed invention in which probes 1 and 2 anneal to each other, forming a loop which quenches a signal such as a fluorescence signal, thereby indicating the absence of the target nucleotide.

Furthermore, while Weston, et al. teach that a loop forms opposite the Hexs (col. 23-24, bridging sentence), the formation of the loop in Weston, et al. is unrelated to any change in signal. In fact, detection in Weston, et al. is carried out by forming a newly synthesized nucleic acid as a result of the hybridization of the two probes to the target sequence and detecting the

newly synthesized nucleic acid directly or indirectly (see col. 4-5, bridging paragraph). To the extent that Weston, et al. mention the use of a label and quencher, Weston, et al. describe an arrangement where the label is on one probe and the quencher is on the other (see col. 10, lines 47-48). This contrasts with Applicant's claimed invention in which the signal from the label is quenched when the loop is formed as recited in step (b) of claim 1, as amended, and both the label and the quencher are on the same probe.

Furthermore, Weston, et al. teach "[w]hen hybridised to target nucleic acid the probes are positioned adjacent to one another and FRET can occur" (col. 10, lines 48-49). That is, the signal is quenched when the two probes are hybridized to the target and adjacent to each other. This is opposite to Applicant's claimed invention. As shown in Figure 1 and recited in amended claim 1, "a higher intensity in the signal of the labeling material [is detected] in the presence of the target, thereby detecting the target nucleic acid". This is opposite to Weston, et al. where the signal is quenched upon binding to the target, not increased. Furthermore, in the presently claimed invention, the two probes are not adjacent as defined by Weston, et al. (see col. 4, third full paragraph).

Applicant's invention differs from Weston, et al. in other respects. In Applicant's claimed invention, the probes 1 and 2 bind together in the absence of the target and the signal is quenched. In the presence of the target, probe 1 binds to the target and the signal from probe 2 is unquenched. This has been clarified by the present amendment which recites "wherein the signal is quenched when the first probe and the second probe are annealed and not quenched when the first probe and the second probe are not annealed, in the presence of the target". Weston, et al. do not teach annealing of the first and second probe, do not teach quenching of a signal and do not teach that a signal is unquenched when the first and second probes are not annealed. Weston, et al. teach that both probes bind to a target in an adjacent manner so that a nucleotide may be synthesized for detection (see col. 4, lines 38-40; cols. 4-5, bridging paragraph). This is totally different from the presently claimed invention.

In Applicant's invention, probe 2 does not bind to the target, in contrast to Weston, et al. where both probes must bind to the target for detection of the target. This is evident from Figure 1 of the present specification and from the following reasoning. If the target did bind to probe 2, the loop would not form, because, by definition, the second region of probe 2 is not

complementary to the target. Weston, et al. do not teach "a second probe comprising a nucleic acid which has a first region that is complementary to at least a portion of the nonspecific region of the first probe, a loop region that does not have a sequence complementary to the first probe, and ***a second region that is complementary to at least a portion of the specific region of the first probe which is complementary to the target sequence***" (emphasis added). In the absence of the loop, there would be no quenching and no change in the signal upon target binding by probe 2.

The claimed invention also differs in the following. In the present invention, in the absence of the target, the second probe binds to the first probe and a signal is quenched. In the presence of the target, the target competes with the second probe to bind to the first probe since both the second probe and the target can bind to the first probe, and a signal increases due to a decrease of binding of the second probe to the first probe (see Example in specification). Therefore, the detection of the signal is competitive. On the other hand, in Weston, et al. a signal is quenched only when the first probe, the second probe, and the target form a complex together. Therefore, the detection of the signal is not competitive.

Probes 2B' and 1B of Weston are not sufficiently complementary for hybridization

Applicant disagrees with the Examiner's statement that two non-contiguous bases can hybridize to each other. The Office Action states that there is no teaching in the specification or claims that says that in order for two sequences to hybridize that they must have an least N amount of complementary base pairs that are contiguous (page 8, first full paragraph).

However, one of ordinary skill in the art would know that hybridization cannot occur when only two bases out of 13 match. This is evidenced by the following calculation.

The melting temperature (T_m) at which 50% of a probe is annealed to its complementary strand is defined by:

$T_{m=} 81.5 + 16.6 \log M + 41 (\%G + \%C) - 500/L - 0.62F$

where

M = molar concentration of monovalent cations

%G or C =the respective fraction of G and C nucleotides in the probe

L = length of annealed product

F = molar concentration of formamide

(see Attachment – HYBRIDIZATION NOTES –Melting and Annealing Temperature)

A standard PCR buffer composition is:

50 mM KCl

10 mM Tris-HCl (pH 8.4-9.0 at 25 °C)

1.5 mM $MgCl_2$

0.01% gelatin or 0.01% Triton X-100

Therefore, M is 61.5 mM (i.e. 0.0615 M) and an oligonucleotide probe with the sequence of A and C as shown in Figure 11C of Weston et al. in a 0.0615 M solution without formamide has a predicted T_m as follows:

$T_m = 81.5 + (16.6 * \log10\ (0.0615)) + (0.41 * 50\%) - (500/2)$

$= 81.5 + 1.2111 + 20.5 - 250$

$= -149.2$

In conclusion, it is clear that the oligonucleotides probe does not hybridize at typical temperature conditions.

In view of Applicants' amendments and arguments, reconsideration and withdrawal of this ground of rejection is respectfully requested.

CONCLUSION

In view of Applicants' amendments to the claims and the foregoing Remarks, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: Dec. 22, 2006

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